Transforming Competent Cells

Perform the following before starting the transformation procedure:

• Equilibrate a water bath to 42°C.

• Warm the vial of LB Medium to room temperature.

• Warm the selective plates in a 37°C incubator for 30 minutes (use one plate for each transformation).

For optimal growth, it is essential that selective plates are prewarmed to 37°C prior to spreading.

Transformation Procedure

1. Thaw, on ice, one vial of freshly made chemically competent E. coli for each transformation.

2. Add 2 µl of the DNA into a vial of cells and mix gently. Do not mix by pipetting up and down.

3. Incubate the vial(s) on ice for 30 minutes.

4. Heat-shock the cells for 30 seconds at 42°C without shaking.

5. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.

6. Add 250 µl of room temperature LB Medium to each vial.

7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.

8. Spread 75 µl of the transformation mix on a prewarmed selective plate. Store the remaining transformation mix at+4°C. Additional cells may be plated out the next day, if desired.

9. Invert the plate(s) and incubate at 37°C. If you are using ampicillin selection, visible colonies should appear within 8 hours, and blue/white screening can be performed after 12 hours. If you are selecting transformants with an antibiotic other than ampicillin, incubate plates overnight.

10. Select overnight-grown colonies and analyze by plasmid isolation, PCR, or sequencing.